UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/779,560	02/09/2001	Marianne Harboe	58982.000002	6162

7590 04/09/2002

Stanislaus Aksman
Hunton & Williams
Suite 1200
1900 K Street, N.W.
Washington, DC 20006

EXAMINER
STEADMAN, DAVID J

ART UNIT	PAPER NUMBER
1652	8

DATE MAILED: 04/09/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/779,560	HARBOE, MARIANNE	
	Examiner	Art Unit	
	David J. Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-34 is/are pending in the application.

4a) Of the above claim(s) 33 and 34 is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-32 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 4 .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Application Status

Claims 1-34 are pending in the application.

Applicants election with traverse of Group I, claims 1-32, drawn to a method of providing a polypeptide preparation having a reduced content of undesired enzymatic side effects in Paper No. 7, filed 02/11/02 is acknowledged.

The examiner requests that applicants provide a copy of all pending claims in subsequent communications.

Election/Restrictions

1. Applicants traverse the restriction requirement on the grounds that the inventions of Groups I and II are related and therefore, the examiner's assertion that the claims of Group I are independent and distinct from the claims of Group II is improper. As stated in the Office action of Paper No. 6, the inventions *are* related, however, they are related as process of making and product made, and therefore, are patentably distinct inventions because the composition of Group II can be made by other methods, e.g., heat denaturation or standard protein purification, rather than by exposure to acidic pH. Therefore, the inventions of Groups I and II are patentably distinct inventions.

The requirement is still deemed proper and is therefore made FINAL.

Claims 33 and 34 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Specification/Informalities

2. The title of the invention is not descriptive. A new title is required that is clearly indicative of the invention to which the claims are directed. The following title is suggested: "Method of Preparing An Aspartic Protease Free of Acid-Labile Enzymatic Activities". See MPEP § 606.01.

3. The specification is objected to because, while descriptions for Figures 1 and 2 have been provided, there is no "Brief Description of the Drawings" heading. It is suggested that applicants insert the phrase "Brief Description of the Drawings" prior to the descriptions of Figures 1 and 2 at page 9 of the specification.

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

5. The term "reduced" in claim 1 (claims 2-32 dependent therefrom) is unclear absent a statement defining to what the level of content of undesired enzymatic side activities is being compared. The term "reduced" is a relative term and the claim should define and clearly state as to what the level of content of undesired enzymatic side activities is being compared (i.e., reduced in comparison to what level undesired enzymatic side activities?).

6. Claim 1 (claims 4-32 dependent therefrom) is confusing in that it is unclear as to whether the desired polypeptide remains stable, i.e., maintains biological activity, or is denatured by the medium. It appears from the specification (for example, page 4, lines 26-29) and claims 2 and 3 that the desired polypeptide maintains biological activity upon treatment of the medium at pH 2.0. It is suggested that applicants clarify the meaning of the claim.

7. Claims 2 and 3 are indefinite in the recitation of "activity of the at least one desired polypetide" as it is unclear as to the recited polypeptide "activity". Claim 1 does not require that the desired polypeptide have *any* activity. The specification discloses the meaning of the term "biologically active" as "any detectable activity that can be detected" and provide examples such as antimicrobial activity, enzyme activity, etc. (page 5 of the instant specification). However, the scope of things encompassed by

this "definition" is vague and it is unclear from the definition of this term what functions of the desired polypeptide applicants intend as the meaning of "activity". It is suggested that the term "activity" be replaced with, for example, "enzymatic activity".

8. Regarding claims 10, 11, 24, 27 (claims 28 and 30-32 dependent therefrom), 29 (claim 30 dependent therefrom), the phrase "including" renders the claims indefinite because it is unclear whether the limitation(s) following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

9. Claims 20, 21 (claims 22-26 dependent therefrom), 27 (claims 28 and 30 dependent therefrom), 29 (claim 30 dependent therefrom), 31, and 32 are indefinite in the recitation of "derived". It is unclear from the specification and the claims as to the derivatives of a medium or an aspartic protease to which applicants refer. It is suggested that applicants clarify the meaning of the term by, for example, deleting the term "derived".

Claim Rejections - 35 USC § 112, First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claim 32 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 32 is directed to a method of producing an aspartic protease wherein the aspartic protease is derived from a naturally produced aspartic protease by addition, deletion, or substitution of one or more amino acids. The specification teaches only two representative species of such aspartic proteases, i.e., an amylase or glucoamylase-prochymosin fusion protein. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being an aspartic protease derived from a naturally produced aspartic protease by

addition, deletion, or substitution of one or more amino acids. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

11. Claims 1-32 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of preparing chymosin with reduced undesired glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease activities by treating a non-acidophilic cell medium comprising said enzymes at a pH of 1.6 to 1.8 for a time sufficient to reduce glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease enzyme activities as compared to untreated glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease enzymes, does not reasonably provide enablement for a method of producing *any* desired polypeptide having reduced content of *any* enzymatic side activities by treating *any* medium with *any* pH of less than 2.0 as encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re* Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 1-32 are so broad as to encompass a method of producing *any* desired polypeptide having reduced content of *any* enzymatic side activities by treating *any* medium with *any* pH of less than 2.0 as encompassed by the claims. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polypeptides, undesired enzyme activities, media, and pH values broadly encompassed by the claims. In this case the disclosure is limited to a method of preparing chymosin with reduced undesired glucoamylase, peptidase, amylase, cellulase,

phosphatase, and protease activities the method comprising treating a non-acidophilic cell medium comprising said enzymes at a pH of 1.6 to 1.8 for a time sufficient to reduce glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease enzyme activities as compared to untreated glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease enzymes.

The expectation of obtaining a desired polypeptide with an ability to maintain activity while an undesired enzyme has reduced activity in any medium at a pH below 2.0 as encompassed by the claims with a reasonable expectation of success in obtaining the desired activity/utility are limited and the result is highly unpredictable.

The specification does not support the broad scope of the claims which encompass a method of producing *any* desired polypeptide having reduced content of *any* enzymatic side activities by treating *any* medium with *any* pH of less than 2.0 as encompassed by the claims because the specification does not establish: (A) a rational and predictable scheme for treating *any* desired polypeptide including enzymes, antibodies, antigens, and pharmaceutically active polypeptides at a pH of less than 2 without affecting desired polypeptide activity, as not all polypeptides as encompassed by the claims are able to maintain activity at a pH of less than 2; (B) methods of treating an undesired polypeptide in any medium at a pH of less than 2 with an expectation of reducing *any* undesired polypeptide enzyme activity, as some polypeptides, particularly those isolated from acidophilic bacteria, will not have reduced enzyme activities in an acidic pH; and (C) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method of producing *any* desired polypeptide having reduced content of *any* enzymatic side activities by treating *any* medium with *any* pH of less than 2.0 as encompassed by the claims. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re* Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is

unnecessarily, and improperly, extensive and undue. See *In re* Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 1-9, 12-22, 25-28, and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laustsen (US Patent 6,080,564) in view of Larsen (WO 95/29999) and Heinsohn (US Patent 5,215,908). Claim 1 is drawn to a method of preparing a desired polypeptide with reduced undesired enzymatic activities by treating a medium at a pH of less than 2.0 for a time sufficient to reduce undesired enzyme activity. Claims 2 and 3 limit the remaining activity of the desired polypeptide after treatment at a pH of less than 2.0. Claims 4, 5, 24, and 26 limit the activity of the undesired enzyme activity. Claims 6, 9-12, and 20-22 limit the source of the medium. Claims 7 and 27 further limit the type of desired polypeptide. Claims 19, 23, 27, and 28 further limit the enzymatic activity of the desired polypeptide. Claim 8 further limits the undesired enzyme activity. Claims 13-17 further limit the pH of the medium for removing undesired enzyme activity. Claim 18 further limits the time sufficient to reduce undesired enzyme activity. Claims 29-32 further limit the source of the desired polypeptide.

Laustsen teaches a method of obtaining a desirable enzyme with inactivated undesired enzyme activities by treatment with low pH (column 1, top). Laustsen teaches that undesirable enzymes may be any enzyme, but are particularly lipases, amylases, cellulases, oxidoreductases, xylanases, isomerases, peptidases, and proteases and may be obtained from plant, animal, or microbial sources (column 2, bottom). Laustsen teaches that desirable enzymes may be any enzyme, but are particularly lipases, amylases, cellulases, oxidoreductases, xylanases, isomerases, peptidases, and proteases and may be

obtained from plant, animal, or microbial sources (column 3, top). Laustsen teaches a method of determining optimal conditions (pH, time, and temperature) for obtaining a desired polypeptide with inactivated undesired enzymatic activities by determining the pH optimum of the desired polypeptide (column 4, top). Laustsen teaches it is advantageous to hold the pH as acidic as possible for a desired polypeptide with an acidic pH optimum (column 4, top). Laustsen teaches that the pH can be adjusted using an inorganic or organic acid (column 4, bottom). Laustsen teaches the time required to inactivate the undesired enzymes can typically range from 20 seconds to up to 2 weeks (column 4, middle). Laustsen teaches the undesired enzyme should be inactivated to a level of at least 1-15 % of the original undesired enzyme activity (column 4, bottom). Laustsen teaches the desired polypeptide should maintain at least 50 % of the original desired polypeptide activity (column 4, bottom). Laustsen provides examples of the disclosed method by treating an Aspergillus medium with low pH, thereby significantly reducing protease activity at a pH of 3.5 (Examples 1, 2, 7, and 8), amylase activity at a pH of 3.5 (Example 4), and cellulase activity at a pH of 2.0 (Example 6) while maintaining desired lipase, cellulase, or catalase activity. Laustsen does not teach chymosin as a desired polypeptide or using a pH of less than 2.0 to reduce undesired enzyme activities.

Larsen teaches that most commercially available milk clotting enzymes, containing primarily chymosin, are obtained by extraction from an animal stomach, e.g., cattle or pig (page 9, top) by a method involving extraction of the tissue, agitation in a volume of water, removal of particulate matter (page 10, middle). Larsen teaches that following the extraction process the pH of the extract is adjusted to as low as 0.5 using inorganic or organic acids in order to convert the inactive chymosin to an active form (page 10, bottom).

Heinsohn teaches that chymosin is industrially produced by fermentation of filamentous fungi that have been genetically modified to express and secrete chymosin (column 1). Heinsohn teaches chromatographic separation of recombinant chymosin from an aqueous mixture of enzymes produced by fermentation of Aspergillus (column 1). The method of Heinsohn involves initially adjusting the pH of the

growth medium to about 2.0 to stop fermentation and cell growth followed by filtering and column chromatography (column 2, bottom).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to practice the method of Laustsen using an Aspergillus host cell expressing inactive chymosin and adjusting the pH of the resulting medium comprising inactive chymosin to 0.5. One would have been motivated to practice the method of Laustsen using an Aspergillus host cell expressing inactive chymosin and adjusting the pH of the medium to 0.5 in order to remove contaminating protease, amylase, and cellulase enzyme activities while holding the pH as acidic as possible for a desired polypeptide as taught by Laustsen, to convert the inactive chymosin to active chymosin as taught by Larsen, and to stop cell growth and fermentation as taught by Heinsohn, all in a single step. One would have a reasonable expectation of success for practicing the method of Laustsen using an Aspergillus host cell expressing inactive chymosin and adjusting the pH of the resulting medium comprising inactive chymosin to 0.5 because of the results of Laustsen, Larsen, and Heinsohn. Therefore, claims 1-9, 12-22, 25-28, and 31, drawn to a method of preparing a desired polypeptide with reduced undesired enzymatic activities by treating a medium at a pH of less than 2.0 for a time sufficient to reduce undesired enzyme activity would have been obvious to one of ordinary skill in the art.

13. Claims 10, 11, 23, 24, 29, 30, and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laustsen in view of Larsen and Heinsohn as applied to claims 1-9, 12-22, 25-28, and 31 above and further in view of Ward (Biotechnol 8:435-440, May 1990). Claims 10 and 11 limit the source of the medium. Claims 23 and 24 limit the desired polypeptide to an aspartic protease expressed as a fusion protein having at least one undesired enzyme activity (claim 23) or glucoamylase activity (claim 24). Claims 29 and 30 limit the species of origin of the desired polypeptide. Claim 32 limits the desired polypeptide to an aspartic protease with addition, deletion, or substitution of one or more amino acids.

Laustsen, Larsen, and Heinsohn disclose the teachings as described above. Neither Laustsen, Larsen, nor Heinsohn teaches or suggests a fusion protein comprising an aspartic protease and having an undesired enzyme activity or glucoamylase activity. Also, Laustsen, Larsen, and Heinsohn do not teach

expression of chymosin using the host cells of claims 10 and 11 or expressing a chymosin from the species of claims 29 and 30.

Ward teaches Escherichia coli, Saccharomyces cerevisiae, and Yarrowia lipolytica have been used successfully as hosts for expression of prochymosin cDNA (page 435, middle). Ward teaches that expression of prochymosin as a fusion in these host cells yielded increased intracellular or extracellular expression (page 435, right). Ward teaches a vector encoding a glucoamylase-bovine prochymosin B fusion protein (page 435, abstract). Ward teaches that following expression and secretion of the fusion protein using Aspergillus awamori as a host, treatment at pH 2.0 released the glucoamylase from the chymosin (page 435, middle). Ward teaches a significant increase in Aspergillus secretion of chymosin when fused to glucoamylase (page 437, Table 2).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to practice the method of Laustsen using an E. coli, S. cerevisiae, or Y. lipolytica host cell expressing chymosin and adjusting the pH of the resulting medium comprising the chymosin protein to 0.5. Also, it would have been obvious to one of ordinary skill in the art at the time of the invention to practice the method of Laustsen using an Aspergillus host cell expressing a glucoamylase-bovine prochymosin B fusion protein and adjusting the pH of the resulting medium comprising the glucoamylase-bovine prochymosin B fusion protein to 0.5. One would have been motivated to practice the method of Laustsen using an E. coli, S. cerevisiae, or Y. lipolytica host cell expressing chymosin and adjusting the pH of the medium to 0.5 because these hosts have been used successfully for chymosin expression as taught by Ward and to remove contaminating protease, amylase, and cellulase enzyme activities while holding the pH as acidic as possible for a desired polypeptide as taught by Laustsen, to convert the inactive chymosin to active chymosin as taught by Larsen, and to stop cell growth and fermentation as taught by Heinsohn. One would have been motivated to practice the method of Laustsen using a host cell expressing a glucoamylase-bovine prochymosin B fusion protein and adjusting the pH of the medium to 0.5 in order to increase the yield of chymosin as taught by Ward and remove contaminating protease, amylase, and cellulase enzyme activities while holding the pH as acidic as possible for a desired

polypeptide as taught by Laustsen, to convert the inactive chymosin to active chymosin as taught by Larsen, and to stop cell growth and fermentation as taught by Heinsohn, all in a single step. One would have a reasonable expectation of success for practicing the method of Laustsen using an E. coli, S. cerevisiae, or Y. lipolytica host cell expressing chymosin and adjusting the pH of the medium to 0.5 because of the results of Laustsen, Larsen, Heinsohn, and Ward. One would have a reasonable expectation of success for practicing the method of Laustsen using an Aspergillus host cell expressing a glucoamylase-bovine prochymosin B fusion protein and adjusting the pH of the resulting medium comprising the glucoamylase-bovine prochymosin B fusion protein to 0.5 because of the results of Laustsen, Larsen, Heinsohn, and Ward. Therefore, claims 10, 11, 23, 24, 29, 30, and 32, drawn to a method of preparing a desired polypeptide with reduced undesired enzymatic activities by treating a medium at a pH of less than 2.0 for a time sufficient to reduce undesired enzyme activity would have been obvious to one of ordinary skill in the art.

Conclusion

14. All claims are rejected.

15. No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The Examiner can normally be reached Monday-Friday from 7:30 am to 2:00 pm and from 3:30 pm to 5:30 pm. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Group is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600